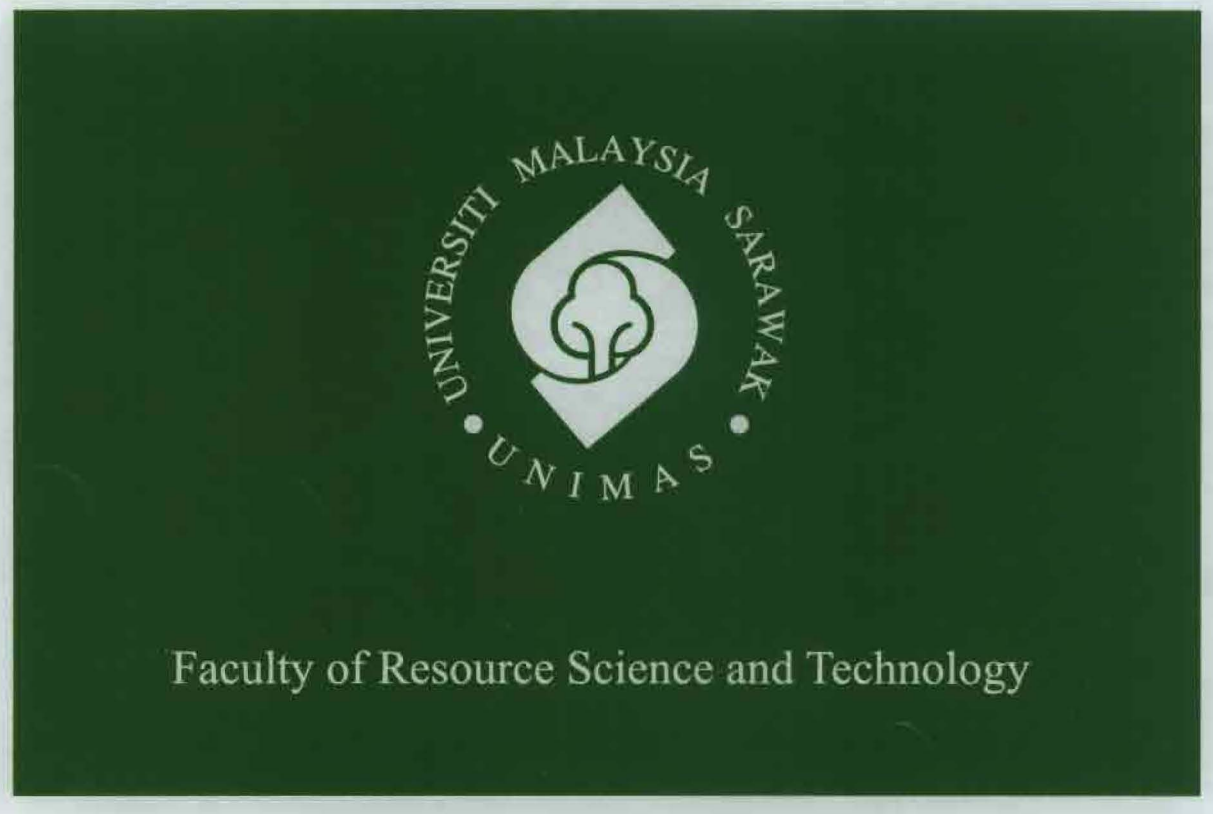

DETECTION OF PATHOGENIC *LEPTOSPIRA* IN ENVIRONMENTAL SOURCES FROM SELECTED URBAN SITES AND NATIONAL PARK IN SARAWAK

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Declaration

I, Choe Sin Pei (35740) hereby declare that the final year project work entitled "Detection of Pathogenic *Leptospira* in Environmental Sources from Selected Urban Sites and National Park in Sarawak" is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

22/6/15

Date submitted

Signature

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List of Abbreviations

DNA	Deoxyribonucleic acid
rDNA	Ribosomal deoxyribonucleic acid
EMJH medium	Ellinghausen-McCullough-Johnson-Harris medium
PCR	Polymerase chain reaction
rpm	Revolution per minute
µL	Microliter
TBE buffer	Tris-borate EDTA buffer

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Detection of Pathogenic *Leptospira* in Environmental Sources from Selected Urban Sites and National Park in Sarawak

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ABSTRACT

Leptospirosis which is caused by pathogenic *Leptospira* had been gazetted as one of the notifiable zoonotic diseases in Malaysia since year 2010. According to the reports by Sarawak State Health Department, based on the cases in 2014, the drastic increase of leptospirosis cases happened in Sarawak had reached 616 cases with 24 deaths. Several recent studies have reported the isolation of pathogenic *Leptospira* from environmental soil and water in recreational parks and selected national service training centres in Malaysia. However, there is very limited information about this disease in water and soils of urban sites, particularly in Sarawak. Thus, this project was carried out to study the occurrence of pathogenic *Leptospira* in environmental sources (water and soil) from urban areas and national park in Kuching, Sarawak. A total of 360 samples (180 soil and 180 water) were collected from Gunung Gading National Park (GGNP) and the residential and commercial area of six urban areas in Kuching. The samples were processed through ultra-pore membrane filtration and then inoculated into EMJH medium followed by the incubation period of 30 days. PCR was conducted to detect pathogenic *Leptospira* by using lipL32-270F/lipL32-692R primers which target on *lipL32* genes. There were 5.6% (20/360) of environmental sources tested positive for pathogenic *Leptospira*. In comparison, detectable leptospital DNA was presented in 0.83% (1/120) environmental sources from national park and 7.9% (19/240) environmental sources from six urban areas. The result demonstrated high prevalence of *Leptopira* in the environmental sources from urban areas than that from national park. Additionally, *Leptospira* presented higher prevalence in soil samples (10%) than that in water samples (1.1%). Further identification of isolates in strain and serovar level is needed to understand the distribution of *Leptospira* in urban sites of Kuching to prevent leptospirosis outbreak.

Keywords: Leptospirosis, PCR, *lipL32* genes

ABSTRAK

Leptospirosis, penyakit yang disebabkan oleh bakteria Leptospira telah diwartakan sebagai salah satu penyakit zoonosis yang wajib dilaporkan di Malaysia sejak tahun 2010. Berdasarkan laporan yang disediakan oleh Jabatan Kesihatan Negeri Sarawak pada tahun 2014, kenaikan kes leptospirosis yang berlaku di Sarawak telah mencapai 616 kes termasuk 24 kes kematian. Kajian tentang pengasingan patogen genus Leptospira daripada sumber alam sekitar seperti sumber air and tanah telah dilaporkan baru-baru ini di kawasan rekresi dan tapak pelatihan Program Latihan Khidmat Negara di Malaysia. Namun, kajian tentang penyebakan penyakit leptospirosis di kawasan bandar masih tidak mencukupi. Dengan itu, projek ini dilaksanakan untuk memerhati dan menjejaki kejadian genus Leptospira dalam sumber alam sekitar di kawasan bandar Kuching dan juga Taman Negara Gunung Gading, Sarawak. Sebanyak 360 sampel telah dikumpul untuk dianalisiskan. Sampel diproses melalui kertas penapisan yang mempunyai ultra-liang dan seterusnya disuntik ke dalam EMJH media untuk proses pengeraman bakteria selama 30 hari. PCR telah dijalankan untuk mengesan kehadiran patogen Leptospira dengan menggunakan primer lipL32-270F/lipL32-692R yang mensasarkan gen lipL32.Sebanyak 5.6% sumber alam sekitar telah dikesan positif untuk patogen Leptospira. 0.83% (1/120) sumber alam sekitar dari Taman Negara telah dikesan mempunyai DNA Leptospira manakala 7.9% (19/240) adalah dari kawasan bandar. Peratusan ini menunjukan kehadiran Leptospira lebih lazim di kawasan bandar daripada di taman negara. Selain itu, bakteria Leptospira lebih banyak dikesan dalam tanah (10%) berbanding dengan air (1.1%). Untuk mengelakkan tercetusnya penyakit leptospirosis, pengenalpastian lanjut tentang jenis dan serovar Leptospira amat diperlukan bagi memahami penyebaran bakteria Leptospira di kawasan bandar Kuching.

Kata Kunci: *Leptospirosis, PCR, lipL32* gen

1.0 Introduction

Leptospirosis had been gazetted as one of the notifiable zoonotic diseases in Malaysia since year 2010 under Prevention and Control of Infectious Diseases Act, 1988 (Sarawak State Health Department, 2014). This endemic infectious disease is caused by a pathogenic bacterium known as *Leptospira* which belongs to the order of Spirochaetales, family of Leptospiraceae and genus of *Leptospira* (Thayaparan *et al.*, 2013). Morphologically, *Leptospira* have thin, spiral body shape with one or two hooked ends as well as active motility. These characteristics are the pri carrier mary observable microscopy characteristics that help to differentiate *Leptospira* from other bacteria particularly when under dark field microscope (Levett, 2001).

Classifications of *Leptospira* are studied based on their serological characteristics and pathogenicity. Until now, approximately 200 studied serovars are discovered in Malaysia (Lim *et al.*, 2011). In order to understand the epidemiology of leptospirosis, based on the pathogenicity, genus *Leptospira* are further organized into three distinct groups which include pathogenic strain, intermediate strain that have unclear pathogenicity, and saprophytic strain (Ricaldi *et al.*, 2012). Leptospirosis is mainly caused by pathogenic strain of *Leptospira* either through direct or indirect transmission. For direct transmission, human may infected with leptospirosis when contact directly with infected animal urine or body fluid. Among those common domestic animals, such as dogs, pigs, cattle and rodents, that have potential to carry and transmit *Leptospira*, rodents have been recognized as the dominant maintenance host of *Leptospira* (Levett, 2001; Thayaparan *et al.*, 2013). On the other hand, indirect association with contaminated environmental sources such as soil and water which has been polluted by rodent urine can also lead to *Leptospira* infection.

Since *Leptospira* has the ability to survive in aerobic condition with optimum growth temperature of 28 °C to 30 °C, the outbreak of leptospirosis in tropical countries may become endemic as environment sources provide favourable condition for the growth of *Leptospira* in the aspect of moisture, pH and temperature (Levett, 2001; Thayaparan *et al.*, 2013). The association between human and environmental sources during human daily activities such as farming, hunting, eco-tourism and swimming, increases the risk to leptospirosis (Thayaparan *et al.*, 2013). According to Alexander *et al.* (1975), there were 29 distinct serovars of *Leptospira* being isolated from natural water and soil in West Malaysia. Among those serovar, the strain of *Leptospira* that most commonly existed in soil and water in Malaysia are Icterohaemorrhagiae serotype mankarsoa, smithii and birkin (Alexander *et al.*, 1975). Besides, study done by Ridzlan *et al.* (2010) in Kelantan and Terengganu, Malaysia particularly in national service training centres showed the presence of *Leptospira* serovar Hebdomadis in water and soil samples.

The occurrence of leptospirosis in Malaysia increased significantly through the centuries since the first cases being discovered by Fletcher in 1925 (Lim *et al.*, 2011). The number of leptospirosis cases in Malaysia raised from 263 cases with 20 deaths in 2004 to 2925 cases with 28 deaths in 2013 (Lim *et al.*, 2011; Lee, 2013). According to the reports by Sarawak State Health Department (2014), based on the cases which had happened in year 2014, the number of leptospirosis cases in Sarawak had reached 616 cases with 24 deaths. Bintulu, Kuching and Miri posed the most number of leptospirosis in 2014 with 141, 113 and 105 cases respectively (Sarawak State Health Department, 2014). The possible reasons contribute to the elevation of leptospirosis are the improper management of water source and garbage in urban area as well as flood (Thayaparan *et al.*, 2013; Benacer *et al.*, 2013b). This creates a favourable environment for animal carrier especially rat to survive. As a result, the

urine excreted from animal carrier contaminates the water and soil. Thus, further preventive measurement has to be taken to prevent the outbreak of leptospirosis in Sarawak.

Due to the limitation of information about the prevalence of *Leptospira* in environmental sources, particularly in Kuching, Sarawak, this study was conducted with the main purpose to detect the occurrence of pathogenic *Leptospira* in water and soil from selected urban area of Kuching and Gunung Gading National Park. The detection of *Leptospira* in environmental sources was performed by using Specific Polymerase Chain Reaction (PCR) assay. The present study observed that the survival of *Leptospira* in water and soil were found to be related to other factors such as pH and temperature.

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2.0 Literature Review

2.1 Taxonomy of *Leptospira*

Different taxonomy systems have been introduced by scientists to classify genus *Leptospira* to ease the understanding of their epidemiology. The classification can be based on their phenotype or genotype. Formerly, by referring to their pathogenicity, genus *Leptospira* was arranged into two distinct species which include *L. interrogans* that comprise of pathogenic strain and *L. biflexa* that comprise of saprophytic strains (Levett, 2001). These two species are further classified into serogroup and serovar by studying their surface antigen through agglutination technique (Mohammed *et al.*, 2011). According to Picardeau (2013), 25 serogroups with more than 300 leptospiral serovars had been studied and recognized. Among these recognized serovars, approximately 60 serovars had been found belong to *L. biflexa*, whereas more than 225 serovars were belonged to *L. interrogans*.

Along with the advancement of technology to determine more genomic information of bacteria, genus *Leptospira* are rearranged genotypically into 20 genomospecies which strengthen the taxonomic foundation of *Leptospira* (Levett, 2001). According to Picardeau (2013), these 20 species consist of nine pathogenic, six saprophytic and five intermediate species as showed in Table 2.1.

Table 2.1. The genomic classification of genus *Leptospira* (Maneewatch *et al.*, 2014; Smythe *et al.*, 2013).

Pathogenic species	Intermediate species	Saprophytic species
L. interrogans	*L. inadai*	*L. biflexa*
L. borgpetersenii	*L. fainei*	*L. meyeri*
L. kirschneri	*L. broomii*	*L. wolbachii*
L. alexanderi	*L. licerasiae*	*L.vanthielii* (genomospecies 3)
L. alstoni (genomospecies 1)	*L. wolffii*	*L.terpstrae* (genomospecies 4)
L. kmetyi		*L. yanagawae* (genomospecies 5)
L. noguchii		
L.santarosai		
L. weilii		

2.2 Characteristic of *Leptospira*

Leptospira is characterised morphologically by its thin spiral shape with 6 to 20 μm long (Bharti *et al.*, 2003). A hook-like shape at one or both end of *Leptospira* can be observed under dark-field microscope. *Leptospira* pose different types of mobility when present in different types of medium. Three types of movement that can be observed in *Leptospira* are rotation around a central axis, progressive movement in the direction of the straight end, and circular motion (Bharti *et al.*, 2003). In semisolid media, *Leptospira* move by bending or flexion motion. Haake (2000) stated that *Leptospira* is an obligate aerobic spirochete that contains features of both Gram-positive and Gram-negative bacteria. Thus, instead of gram-staining, microscopy technique and further biochemical analysis are more suitable to determine its presence (Hawley *et al.*, 2013). According to Muhammed *et al.* (2011), *Leptospira* cannot live in drought or hypertonicticity, but they sustain alkali condition up to pH 7.8.

Pathogenic *L. interrogans* live in renal tubules of host and can cause disease in animal and human whereas saprophytic strain are free living bacteria which normally found in wet environmental sources such as water and soil and do not cause disease (World Health Organisation, 2003). According to Levett (2001), the ability of *L. biflexa* to survive at 13 °C and in the medium with 8-azaguanine is one of the characteristics to differentiate these two species.

2.3 Transmission mode of *Leptospira*

According to World Health Organisation (2003), leptospiral infections are acquired by two different transmission modes which include direct and indirect contact between human and urine or other body fluids of infected animal that has been contaminated by viable *Leptospira*. Pathogenic *Leptospira* infect human through wound on skin, mucous membranes and waterlogged skin (World Health Organisation, 2003). This can happen when human contacts

with water or soil that contain *Leptospira*-contaminated urine. Rodents especially rats are the primary hosts of *Leptospira*. A study carried out in Kuala Lumpur by Benacer *et al.* (2013a) showed that *Rattus rattus* was the dominant carrier of *Leptospira interrogans* and *Leptospira borgpetersenii* which poses high pathogenicity.

2.4 Epidemiology

Leptospirosis cases are widespread. It is often being reported in tropical climates country. According to Thayaparan *et al.* (2013), this happens because of the ability of *Leptospira* to survive and live in a wide range of animal especially domestic animal and human. Other than that, the ability of *Leptospira* to live outside the host as long as the environment provides favourable condition also contributes to higher incidence of leptospirosis (Thayaparan *et al.*, 2013). Based on the study carried out by Tan (1970), leptospirosis is an occupational disease (as cited in El Jalii & Bahaman, 2004). General laborers and rubber estate workers were placed on top of name list due to their frequent exposure to environmental sources (Ungku Omar, 1967 as cited in El Jalii & Bahaman, 2004). Serovar canicola, icterohemorrhagiae, pyrogenes, hebdadis and autumnalis were isolated from human cases based on the studies done by Bahaman and Ibrahim (1987). Besides, recreational activities especially water sport which took place at public environment such as lake, stream, and pond were proved to be a potential area for the spread of leptospirosis (Victoriano *et al.*, 2009). According to Sapian *et al.* (2012), the outbreak of leptospirosis in public natural recreational forest with waterfall and stream at Hutan Lipur Lubuk Yu, Maran, in Pahang, Malaysia had gained public concern. The isolation of serovar Hebdomadis from water and soil samples in National Service Training Centers in Kelantan and Terengganu, Malaysia by Ridzlan *et al.* (2010) also raised the concern.

2.5 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) which was first introduced by Kary Mullis in 1983 has become a revolutionary technique that forms the cornerstone of the study of molecular biology and genetics nowadays (Bartlett & Stirling, 2003). There are several studies done by researchers to detect and differentiate the species of *Leptospira* by using PCR technique (Bal *et al.*, 1994; Bourhy *et al.*, 2011; Kositanont *et al.*, 2007; Murgia *et al.*, 1997; Yusouri *et al.*, 2013). For early detection, Bal *et al.* (1994) showed that PCR is one of the promising approaches to detect *Leptospira* in urine. To fully accomplish the use of PCR technique in the early detection of *Leptospira*, different types of PCR which have potential to detect *Leptospira* effectively were studied. Bourhy *et al.* (2011) compared the sensitivity and specificity of four quantitative real-time PCR assay to detect *Leptospira*. Other than that, Kositanont *et al.*(2007) also tested the effetiveness of PCR to detect *Leptospira* in blood sample. The design of primer specific for pathogenic and saprophytic *Leptospira* in water was introduced by Murgia *et al.* (1997). In the case of identifying pathogenic *Leptospira*, the primer used are more specifically targeting on genes such as *lipL21, lipL32, lipL41, ligA and ligB* (Thaipadunpanit *et al.*, 2011). Due to the rapidity, sensitivity and specificity of PCR technique, this approach permits the early detection of *Leptospira* (Benacer *et al.*, 2013a).

3.0 Materials and Methods

3.1 Materials and apparatus

The list of materials used in this study was stated in Appendix 1.

3.2 Study sites

In this study, the sampling trips were carried out during October 2014 to January 2015. Soil and water samples were collected from Gunung Gading National Park and 6 urban sites named Camp MIKE, Medan Niaga Satok, Kampung Gita, Kampung Tupong, Hui Sing as well as Kampung Paya Mebi. The locations of all seven sampling sites are indicated in maps in Figure 3.1 and Figure 3.2.

The sampling trip in Gunung Gading National Park (GGNP) was conducted from 21 to 25 October 2014. The average temperature in this area ranged from 26.0 °C to 33.5 °C with humidity range of 73.4 to 99.9. A total of 60 soil and 60 water samples were collected from the national park which has recreational value as tourism site and hiking area. Sixty water samples were collected randomly from waterfall, stream and effluent drain water, while 60 soil samples were also collected randomly from the forestry area which is normally visited by tourists for hiking activities.

As for the urban area, the samples collections in these 6 areas: Medan Niaga Satok, Camp MIKE, Kampung Gita, Kampung Tupong, Hui Sing and Kampung Paya Mebi were conducted during December 2014 and January 2015. These locations were situated in or near the urban area in Kuching. Medan Niaga Satok is the biggest market in Kuching whereas Hui Sing is the commercialize area in Kuching town that have popular hawker centre. These areas are located near residential area. On the other hand, Kampung Gita,

Kampung Tupong and Kampung Paya Mebi are the populated residential urban sites in Kuching. Camp Mike is situated at the suburban area to the north-east direction of Kuching town. It is an experiential learning camp that located at the foothills of Mount Serapi in Matang, Kuching. Twenty soil and 20 water samples were collected from each of the mentioned sampling sites. The targeted sampling locations were chosen randomly which included streams, effluent drain and puddles near residential area and market as well as the soil area which are expected to be infected by rats. As a total, 360 samples were collected from all 7 sampling sites with 180 soil samples and 180 water samples as summarised in Table 3.1.

Table 3.1. Total number of environmental samples collected from seven sampling sites.

Sampling Site	**Water Source**	**Soil Source**	**Number of Samples collected**		**Total**
			Soil	**Water**	
National Park:					
GGNP	Waterfall, stream, drain	Forestry and hiking area	60	60	120
Urban Area:					
Camp MIKE	Manmade pond	Forestry and residential area	20	20	40
Medan Niaga Satok	Stream	Commercial soil area	20	20	40
Kampung Gita	Stream	Residential soil area	20	20	40
Kampung Tupong	Stream	Residential soil area	20	20	40
Hui Sing	Drain	Residential soil area	20	20	40
Kampung Paya Mebi	Drain, puddles	Residential soil area	20	20	40

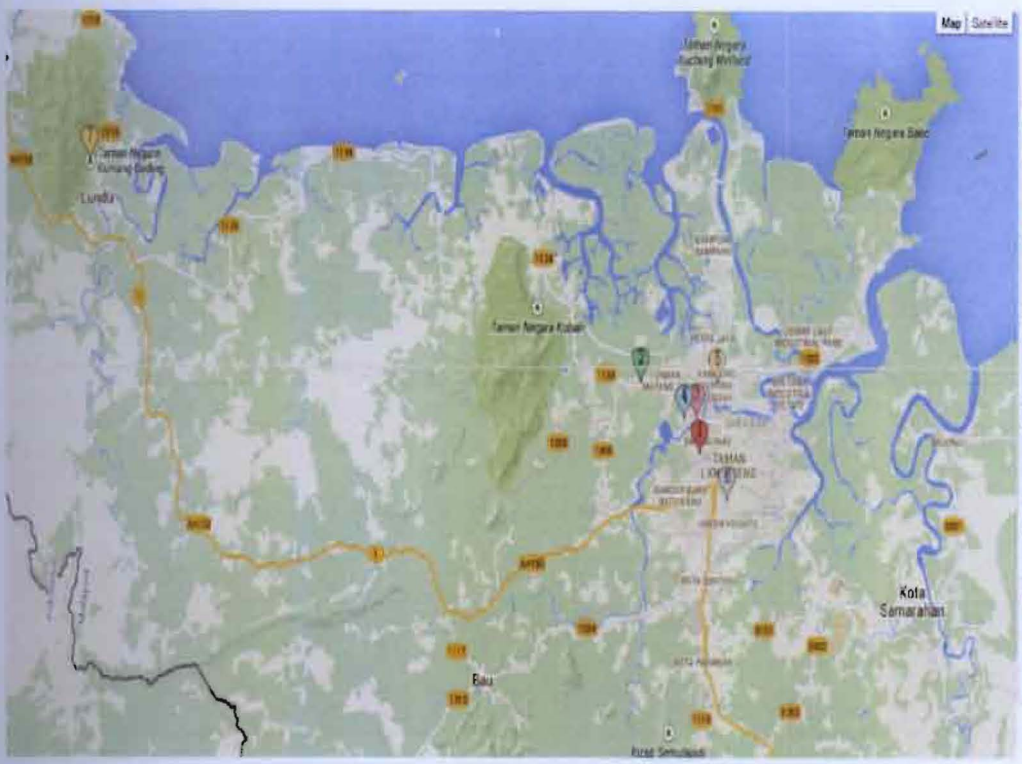

Figure 3.1. The location of seven sampling sites that had been visited (Google map, 2015).

Figure 3.2. The location of six sampling urban sites in Kuching, Sarawak (Google Map, 2015).

3.3 Sample collection

Water and soil samples were collected as described by Henry and Johnson (1978) with some modifications. Approximately 50 mL of water samples were collected from waterfall, stream and drain. As for soil samples, approximately 20 g of topsoil were collected from the wet and shaded area and placed into 50 mL Falcon tubes. The temperature and pH of samples was recorded on the spot using waterproof pH pen model pH100 (Extech, USA) while collecting the samples at the site. The humidity and temperature of environment were also recorded by using humidity/temperature pen (Extech, USA).

3.4 Sample processing

In this study, Ellinghausen-Mc-Cullough-Johnson-Harris (EMJH) medium was prepared to cultivate *Leptospira*. A total of 1 L liquid EMJH culture medium was prepared by adding 100 mL supplement of *Leptospira* Enrichment EMJH (Difco BD, USA) into 900 mL sterile liquid EMJH medium base (Difco BD, USA). 0.1 g of 5-fluorouracil (Merck, Darmstadt, Germany) was added into the medium to minimize bacterial contamination. After preparation of 1 L EMJH culture media, 10 mL of the media was poured into each 15 mL Falcon tubes for further inoculation of processed samples.

The collected water and soil samples were processed as described by Ridzlan *et al.* (2010). Water samples were processed by filtering it through sterile membrane filter with pore size of 0.22 µm. One milliliter of filtered water samples were then inoculated into liquid EMJH culture medium as modified by Johnson and Harris (1967). As for soil samples (20 g), sterile distilled water was prepared and the soil samples were then soaked in the distilled water at three times the volume of the sample in sterile Falcon tube. The soil and distilled water were mixed by shaking vigorously and then allowed to be settled down for 15 minutes. Sterile filter membrane with pore size of 0.22 µm was used to filter the

water suspension. Then, 1 mL of the filtered water was inoculated into liquid EMJH culture medium. All the inoculated media were incubated aerobically for 30 days at room temperature in dark condition.

3.5 Polymerase Chain Reaction

3.5.1 Preparation of DNA template

DNA extraction of 30-day incubated cultures was carried by using Wizard™ Genomic DNA purification Kit (Promega, Madison, WI, USA). 1.5 mL of culture was transferred aseptically into 2 mL microcentrifuge tube and then centrifuged for 5 minutes at 10700 rpm. Supernatant was removed. After that, 600 µL of Nuclei Lysis Solution was added into the tube and vortexed vigorously. The mixture was incubated at 80 °C for 5 minutes. Then, 3 µL of RNase solution was added for cell lysate and incubated in 37 °C for 30 minutes. Two hundred µL of protein precipitation solution was added. The mixture was vortexed vigorously and then centrifuged at 10700 rpm for 3 minutes. The supernatant was transferred into new 1.5 mL microcentrifuge tubes which were previously added with 600 µL room temperature isopropanol. The new mixture was mixed gently by inversion and then centrifuged at 10700 rpm for 2 minutes. The supernatant was discarded and the tube was drained on clean absorbant paper. After the tube was dried, 600 µL of 70% ethanol was added into it and centrifuged at 10700 rpm for 2 minutes. The supernatant was removed and the tube was allowed for air dry for 15 minutes. Once it was dried, 100 µL DNA rehydration solution was added into the tubes. DNA template was incubated at 4 °C overnight. Lastly, DNA was stored at -20 °C for PCR analysis.

3.5.2 Confirmation of pathogenic *Leptospira*

The protocol of PCR reaction was performed according to Benacer *et al.* (2013b) using a total volume of 25 µL PCR components which include 5 X Green buffer, 25 mM $MgCl_2$, 0.5 µL dNTP's, 1 µL of each forward and reverse primer, 0.25 µL of *GoTaq* polymerase DNA (Promega) and 5 µL of DNA template.

The designed primer used in PCR analysis for the detection of pathogenic *Leptospira* was lipL32-270F/ lipL32-692R primer which target on *lipL32* gene. The oligonucleotide sequence of primer and its product size was shown in Table 3.1. The PCR analysis was conducted with PCR amplification condition consisted of initial denaturation at 95 °C for 2 minutes, 35 cycles each of denaturing at 95 °C, 1 minute, annealing at 55 °C, 30 seconds and extension at 72 °C, 1 minute. For the final extension, the condition was 72 °C for 5 minutes.

Table 3.2. The oligonucleotide sequence of primer lipL32-270F/ lipL32-692R and the product size.

Target	Target gene	Primer	Sequence (5'-3')	Product size (bp)	Reference
Pathogenic *Leptospira*	*LipL*32	lipL32-270F	CGCTGAAATGGGAGTTCGTATGATT	423 bp	Vein *et al*, 2012
		lipL32-692R	CCAACAGATGCAACGAAAGATCCTTT		

3.6 Agarose Gel Electrophoresis

The PCR products were subjected to electrophoresis using 2% agarose gel (Promega) in 1 X TBE buffer at voltage of 90 V for 1 hour and 15 minutes. Ethidium bromide was used to strain the gel for further observation and analysis through photography under UV trans illuminator (SigmaAldrich, Germany).

4.0 Results

Upon the incubation period of 30 days, some EMJH media inoculated with samples became turbid. Yellowish-white deposits were observed at the bottom of EMJH culture media. Samples with this observation might due to the growth of *Leptospira*. Pathogenic characteristic of *Leptospira* was further confirmed by using PCR analysis. However, some cultures were found to be contaminated with the presence of black colour precipitates as shown in the photo in Appendix 2.

The presence of pathogenic *Leptospira* was confirmed through PCR analysis by using lipL32-270F/ lipL32-692R primer which target specifically on *lip*L32 gene. 423 bp amplicon bands were visualized on 2% agarose gel under UV light after run through electrophoresis. From the environmental samples collected from seven sampling sites, 5.6% (20/360) pathogenic leptospiral cultures were detected. The visualization of bands for 20 positive samples and 4 randomly chose negative samples (GGS51, STKS07, KPMS14 and HSS15) were shown on agarose gel in Figure 4.1 and Figure 4.2.

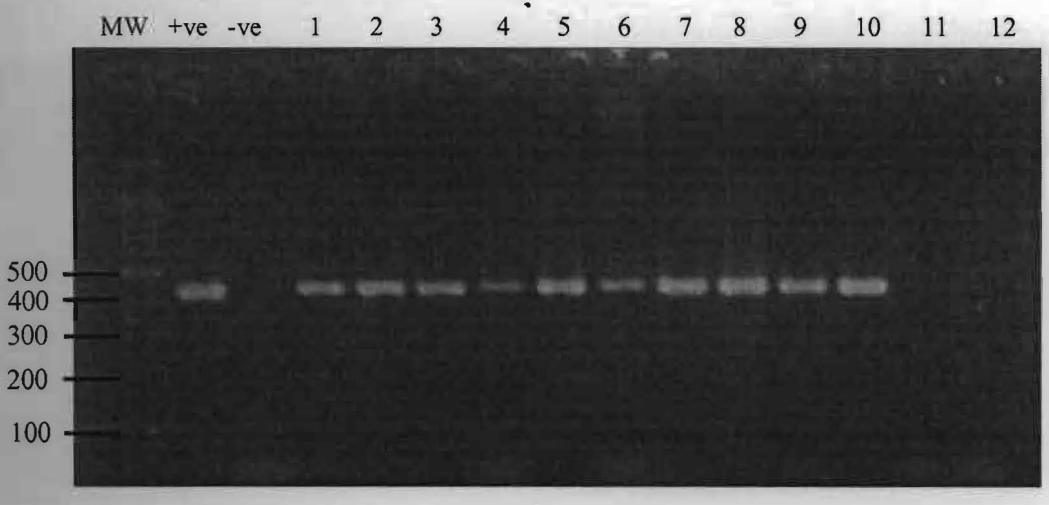

Figure 4.1. The confirmation of pathogenic *Leptospira* on isolates by PCR array.
MW: Molecular marker with 100bp; +ve: positive control; -ve: negative control; Lane: 1, GGW16; 2, CMW14; 3, STKS03; 4, STKS04; 5, STKS05; 6, STKS06; 7, STKS08; 8, STKS09; 9, STKS10; 10 STKS13; 11, GGS51; 12, STKS07

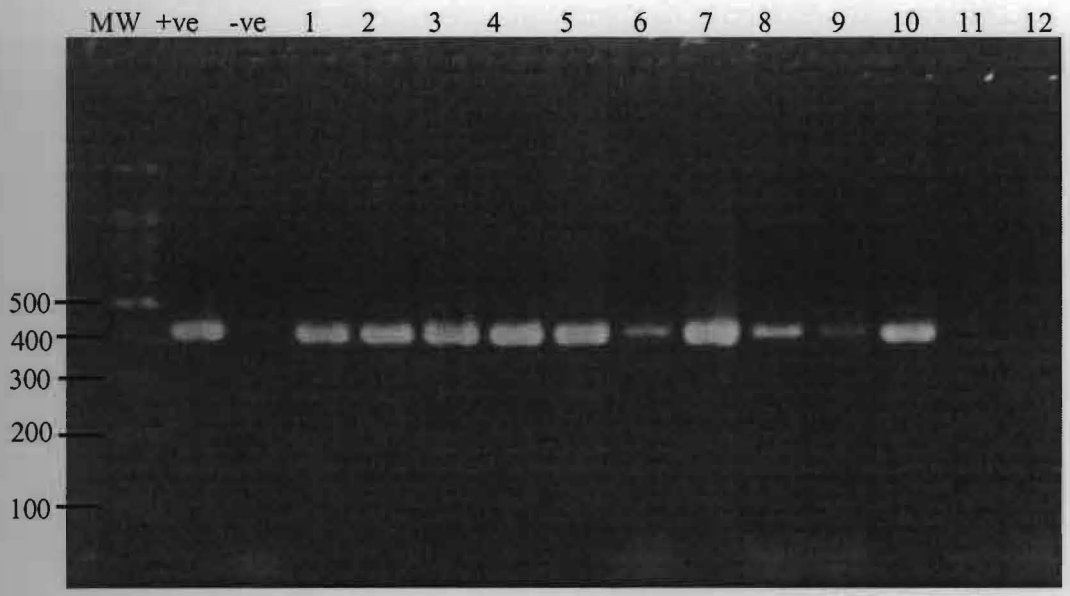

Figure 4.2. Detection of pathogenic *Leptospira* on isolates by PCR array.
MW: Molecular marker with 100bp; +ve: positive control; -ve: negative control; Lane: 1, STKS19; 2, KGS08; 3, KTS05; 4, KTS14; 5, KTS19; 6, HSS07; 7, HSS10; 8, HSS11; 9, KPMS03; 10, KPMS11; 11, KPMS14; 12, HSS15

0.83% (1/120) positive leptospiral isolates were detected from environmental sources Gunung Gading National Park and 7.9% (19/240) positive leptospiral isolates were obtained from urban sites by referring to 120 total samples (60 soil and 60 water) from Gunung Gading National Park (GGNP) and 240 total samples (120 soil and 120 water) from six urban sites. The details of samples collected were summarized in Table 4.1. Among six urban sampling sites, the highest number of positive leptospiral samples was detected in Medan Niaga Satok, with the percentage of 45 % (9/20) of the total number of positive pathogenic leptospiral samples collected.

Apart from that, the PCR result demonstrated the occurrence of *Leptospira* is much frequent in soil than water samples. 1.1% (2/180) positive water samples were detected. As for soil samples, 10% (18/180) positives soil samples were detected through PCR assay. The number of positive leptospiral samples associated with 7 different sampling sites and their average pH as well as average temperature value was summarized in Table 4.2.